

Section II (Amendments to the Claims)

Please amend claims 1, 4, 7, 9, 13, 15 and 46 as set out in the following listing of the claims of the application.

Please cancel claims 18-45 and 50-61, without prejudice.

1. (Currently amended) A nucleic acid construct encoding a fusion protein, wherein the construct comprises a coding sequence for a protein of interest and a coding sequence for a subtilisin prodomain protein, wherein the fusion protein comprises the protein of interest operatively linked to the C-terminus of the subtilisin prodomain protein, wherein the subtilisin prodomain protein binds to a subtilisin or a variant thereof with a Kd of 10 nM or less to form a stable complex, ~~and~~ wherein the subtilisin ~~variant retains the activity of subtilisin or variant thereof is effective to cleave the protein of interest from the subtilisin prodomain protein, and wherein the subtilisin prodomain protein remains bound to the subtilisin or variant thereof following cleavage of the protein of interest.~~

2. (Cancelled)

3. (Previously presented) The nucleic acid construct according to claim 1, wherein the subtilisin prodomain protein further comprises one or more amino acid substitutions that increase binding affinity for subtilisin or a variant thereof, as compared to the subtilisin prodomain protein with no substitutions.

4. (Currently amended) The nucleic acid construct according to claim 1, wherein the subtilisin prodomain protein comprises a variant of SEQ ID NO: [11]₂, wherein the variant comprises a substitution at one or more of positions P1-P4 wherein the substitution comprises any of F or Y substituted for P4, any amino acid residue substituted for P3, A or S substituted for P2 and M, F, Y H, or L substituted for P1.

5. (Cancelled)

6. (Previously presented) The nucleic acid construct according to claim 1, wherein the subtilisin prodomain protein comprises substitutions of amino acid residues F or Y for P4, any amino acid residue for P3, A or S for P2 and M, F, Y, H, or L for P1 at the C-terminal end.

7. (Currently amended) A fusion protein comprising a target protein operatively linked to the C-terminus of a subtilisin prodomain protein, wherein the subtilisin prodomain protein is modified to exhibit an increased affinity for subtilisin or a variant thereof, as compared to the unmodified subtilisin prodomain protein, and wherein the subtilisin variant retains the activity of subtilisin or variant thereof is effective to cleave the target protein from the subtilisin prodomain protein, and wherein the subtilisin prodomain protein remains bound to the subtilisin or variant thereof following cleavage of the target protein.

8. (Cancelled)

9. (Currently amended) The fusion protein according to claim 7, wherein the subtilisin prodomain protein comprises substitution of amino acids P4-P1 with the amino acid sequence FKAM (SEQ ID NO: 10).

10. (Previously presented) The fusion protein according to claim 7, wherein the subtilisin prodomain protein comprises the amino acid sequence E E D K L (F/Y) Q S (M/L/Y) (SEQ ID NO: 7).

11. (Previously Presented) The fusion protein according to claim 7, wherein the target protein is staphylococcal Protein AB domain; Protein AB mutant A219; Streptococcal protein GB domain; Streptococcal protein Ga domain; Protein GB mutant G311; E. coli hypothetical Yab; Bovine a-subunit of transducin; M. thermotrophicus CDC6; streptavidin; avidin; Taq polymerase; alkaline phosphatase; RNase; DNase; restriction enzymes; peroxidases; endo-1, 4-beta glucanase; endo-1, 3-beta-glucanase; chitinases; beta and alpha glucosidases; beta and alpha glucuronidases; amylase; glucosyl-transferases; phospho-transferases; chloramphenicol-acetyl-transferase; beta-lactamase; luciferase; esterases; lipases; proteases; bacteriocines; antibiotics; enzyme inhibitors; growth factors; hormones; receptors; membrane proteins; nuclear proteins; transcriptional factors; translational factors or nucleic acid modifying enzymes.

12. (Previously presented) A DNA construct for the preparation of a fusion protein, wherein the construct comprises a coding sequence of a protein of interest and a DNA sequence encoding a subtilisin binding protein which binds to subtilisin with a Kd of 10 nM or less.

13. (Currently amended) A method for the production of a subtilisin binding fusion protein, the method comprising: providing a nucleic acid construct encoding a fusion protein wherein the

fusion protein comprises a protein of interest operatively linked to the C-terminus of a subtilisin prodomain protein ~~operatively linked to a second protein of interest~~, wherein the subtilisin prodomain protein is modified to bind subtilisin or a variant thereof with increased affinity as compared to an unmodified subtilisin prodomain protein, and wherein the subtilisin variant retains the activity of subtilisin or variant thereof is effective to cleave the protein of interest from the prodomain protein, and wherein the subtilisin prodomain protein remains bound to the subtilisin or variant thereof following cleavage of the protein of interest; transfecting a host cell with the nucleic acid construct; and culturing the transformed host cell under conditions suitable for expression of the fusion protein.

14. (Cancelled)

15. (Currently amended) The method according to claim 13, wherein the subtilisin prodomain is modified by replacing the P4 through P1 amino acids with FKAM (SEQ ID NO: 10), FKAY (SEQ ID NO: 11) or FKAF (SEQ ID NO: 12).

16. (Currently amended) The method according to claim 15, wherein the ~~second~~ protein of interest is staphylococcal Protein AB domain; Protein AB mutant A219; Streptococcal protein GB domain; Streptococcal protein Ga domain; Protein GB mutant G311; E. coli hypothetical Yab; Bovine α -subunit of transducin; M. thermotrophicus CDC6; streptavidin; avidin; Taq polymerase; alkaline phosphatase; RNase; DNase; restriction enzymes; peroxidases; endo-1, 4-beta glucanase; endo-1, 3-beta-glucanase; chitinases; beta and alpha glucosidases; beta and alpha glucuronidases; amylase; glucosyl-transferases; phospho-transferases; chloramphenicol-acetyl-transferase; beta-lactamase; luciferase; esterases; lipases; proteases; bacteriocines; antibiotics; enzyme inhibitors; growth factors; hormones; receptors; membrane proteins; nuclear proteins; transcriptional factors; translational factors or nucleic acid modifying enzymes.

17. (Original) The method according to claim 13, wherein the host cells includes cells from, Escherichia coli, Bacillus, Salmonella, Pseudomonas, Saccharomyces cerevisiae, Pichia pastoris, Kluyveromyces, Candida, Schizosaccharomyces; or CHO cells.

18-45. (Cancelled)

46. (Currently amended) A nucleic acid construct encoding a fusion protein, wherein the construct comprises a coding sequence for a protein of interest and a coding sequence for a second protein, wherein the second protein binds to a subtilisin or a variant thereof with a K_d of 10 nM or less to form a stable complex, and wherein the subtilisin ~~variant retains the activity of subtilisin or variant thereof~~ is effective to cleave the protein of interest from the second protein, and wherein the second protein remains bound to the subtilisin or variant thereof following cleavage of the protein of interest.

47. (Previously presented) A nucleic acid construct according to claim 46, wherein the fusion protein comprises the protein of interest linked to the second protein by a peptide bond and wherein the subtilisin hydrolyzes the peptide bond.

48. (Previously presented) A nucleic acid construct according to claim 46, wherein P1, P2 and P4 amino acids of the second protein generate affinity for S1, S2 and S4 binding pockets of the subtilisin or a variant thereof .

49. (Previously Presented) A nucleic acid construct according to claim 48, wherein the second protein comprises amino acid residues F or Y at the P4 position, any amino acid residue at the P3 position, A, S, V, or T at the P2 position and M, F, Y, H, or L at the P1 position.

50-62. (Cancelled)